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Expression of the FUS domain restores liposarcoma development in CHOP transgenic mice

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Fusion proteins created by chromosomal abnormalities are key components of mesenchymal cancer development. The most common chromosomal translocation in liposarcomas, t(12;16)(q13;p11), creates the *FUS–CHOP* fusion gene. In the past, we generated FUS–CHOP and CHOP transgenic mice and have shown that while FUS–CHOP transgenic develop liposarcomas, mice expressing CHOP, which lacks the FUS domain, display essentially normal white adipose tissue (WAT) development, suggesting that the FUS domain of FUS–CHOP plays a specific and critical role in the pathogenesis of liposarcoma. To test the significance of FUS and CHOP domain interactions within a living mouse, we generated mice expressing the FUS domain and crossed them with CHOP-transgenic mice to generate double-transgenic FUSxCHOP animals. Here we report that expression of the FUS domain restores liposarcoma development in CHOP-transgenic mice. Our results provide genetic evidence that FUS and CHOP domains function *in trans* for the mutual restoration of liposarcoma. These results identify a new mechanism of tumor-associated fusion genes and might have impact beyond myxoid liposarcoma.
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Introduction

Liposarcoma is the most common soft tissue malignancy in adults accounting for at least 20% of all sarcomas in this age group (Chang *et al*., 1989; Mack, 1995). The myxoid/round cell liposarcomas exhibited the characteristic t(12;16)(q13;p11) translocation (Knight *et al*., 1995). The molecular characterization of this translocation revealed a fusion between the *CHOP* gene and a gene called *TLS/FUS* (Crozat *et al*., 1993; Rabbitts *et al*., 1993), resulting in the RNA-binding domain of TLS/FUS being replaced by the basic leucine zipper domain of CHOP, which confers the ability to form protein dimers (Ron and Habener, 1992; Crozat *et al*., 1993). The *TLS/FUS* gene has also been shown to fuse with the gene for Ets-like protein ERG as a result of the translocation t(16;21)(p11;q22) in some patients with acute nonlymphoblastic leukemia (Shimizu *et al*., 1993; Ichikawa *et al*., 1994; Panagopoulos *et al*., 1994, 1995). The portion of TLS/FUS that is present in the TLS/FUS–CHOP and FUS–ERG fusion proteins is similar and this part has been shown to be an autonomous transcriptional activation domain (Prasad *et al*., 1994; Sánchez-García and Rabbitts, 1994; Zinszner *et al*., 1994). In the FUS–CHOP fusion, transcriptional activation is therefore specifically conferred on the chimeric protein by the *FUS* segment after the translocation event (Sánchez-García and Rabbitts, 1994). *In vitro*, the transforming effects of *FUS–CHOP* have been demonstrated in fibroblasts (Pérez-Losada *et al*., 2000a), but curiously not in 3T3–L1 cells under conditions expected to yield oncogenic effects (Zinszner *et al*., 1994), suggesting that the function of FUS–CHOP is influenced by the cell environment *in vitro*. *In vivo*, mice expressing FUS–CHOP develop liposarcomas (Pérez-Losada *et al*., 2000a). Whereas interference with the normal process of differentiation may contribute to the oncogenic potential of FUS–CHOP fusion protein, it is a property that they share with the nontransforming germline CHOP gene product (Zinszner *et al*., 1994; Batchvarova *et al*., 1995). CHOP expression is tightly regulated. Under normal conditions the gene is repressed, and CHOP mRNA and protein are absent from cells (Fornace *et al*., 1989; Price and Calderwood, 1992; Carlson *et al*., 1993; Wang *et al*., 1996). In contrast with CHOP, FUS is a constitutively activated gene (Aman *et al*., 1996). These observations support a model whereby the FUS–CHOP fusion gene repre-

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sents a gain-of-function mutation of CHOP that deregulate both gene expression and protein activity. However, mice expressing high levels of CHOP, which lacks the FUS domain, display normal white adipose tissue (WAT) development (Pérez-Losada *et al*., 2000b), suggesting that the FUS component of the fusion protein acts an autonomous domain which plays a specific and critical role in the pathogenesis of liposarcoma. To test the significance of FUS and CHOP domain interactions within a living mouse, we generated mice expressing the FUS domain and crossed them with CHOP-transgenic mice to generate double-transgenic FUSxCHOP animals. Here we report that expression of the FUS domain restores liposarcoma development in CHOP-transgenic mice. Our results provide genetic evidence that FUS and CHOP domains function *in trans* for the mutual restoration of liposarcoma and support a model whereby the *FUS–CHOP* fusion gene represents a gain-of-function mutation of both FUS and CHOP. This novel molecular mechanism may be responsible for the development of these and other related solid tumors.

Results

Generation of transgenic mice expressing an altered form of FUS–CHOP protein lacking the CHOP domain

We previously developed a mouse model of liposarcoma by expressing FUS–CHOP from the elongation factor 1α (EF-1α) promoter in murine mesenchymal stem cells (Pérez-Losada *et al*., 2000b). In order to understand *in vivo* the role of FUS–CHOP in the pathogenesis of liposarcoma, we have also generated transgenic mice expressing two altered forms of the FUS–CHOP protein (Pérez-Losada *et al*., 2000a). The first truncated protein (CHOP) which lacks the FUS domain did not affect WAT development *in vivo*. The second altered form of FUS–CHOP, created by the in frame fusion of the FUS domain to the carboxy end of CHOP (CHOP–FUS), induced liposarcomas in transgenic mice indicating that the FUS domain of FUS–CHOP plays a specific and critical role in the pathogenesis of liposarcoma (Pérez-Losada *et al*., 2000b). To test the significance of FUS and CHOP domain interactions within a living mouse, we have now generated mice expressing the FUS domain, a truncated FUS–CHOP protein lacking the CHOP domain, to investigate the contribution of the FUS domain to the genesis of liposarcoma *in vivo* (Figure 1a). *In vitro* this truncated protein (FUS) is able to activate transcription (Prasad *et al*., 1994; Sánchez-García and Rabbitts, 1994; Zinszner *et al*., 1994).

In order to examine the direct consequences of *FUS* expression *in vivo* we follow the same strategy than we previously used for FUS–CHOP (Pérez-Losada *et al*., 2000a). Therefore, *FUS* cDNA was cloned downstream from the EF-1α promoter (EF–FUS) to direct expression to all tissues (Figure 1b) and injected into C57BL/6xCBA fertilized eggs. Tissue expression of the EF–FUS transgene was demonstrated by Western blot analysis with an 9E10 monoclonal antibody (Figure 1c).

WAT development is normal in EF-FUS transgenic mice

Cohorts of transgenic mice were generated to analyse the effect of the *FUS* gene. FUS transgenic mice developed healthy, were fertile and none of the FUS transgenic mice developed liposarcomas in up to 24 months of observation. This result is similar to CHOP transgenic mice (Pérez-Losada *et al*., 2000b) and contrasts with our observations in the FUS–CHOP transgenic mice which develop liposarcomas (Pérez-Losada *et al*., 2000a). Although liposarcomas did not develop in the FUS transgenic mice, we investigated whether the FUS protein altered white adipose tissue (WAT) development in these mice. The histopathological analyses of the white adipose depots in FUS transgenic mice did not evidence any pathological change within the terminally differentiated adipocytes (Figure 2). On the contrary, FUS transgenic mice had a normal architecture of the tissue and we did not observe any shift in the WAT toward immature in the FUS transgenic mice.

Double-transgenic FUSxCHOP mice develop liposarcomas

We next investigated the significance of FUS and CHOP domain interactions within a living mouse and crossed mice expressing the FUS domain with CHOP-transgenic mice (Pérez-Losada *et al*., 2000b) to generate double-transgenic FUSxCHOP animals (Figure 1c). Uniformly, male and female double-transgenic FUSxCHOP animals showed the same symptoms beginning as early as 8–12 weeks of age with increasing signs over time leading to death by 12 months of age. At the time of autopsy, these animals had developed palpable masses involving the adipose tissues, which, upon dissection, revealed white adipose pads 4–100-fold normal size. All these animals had visible masses as early as 8–10 weeks. In addition, some animals never developed palpable masses but showed microscopic tumor upon dissection when they were sacrified at 12 weeks of age. This examination is consistent with adipose tissue disease. However, no tumors of other tissues were found in these double-transgenic FUSxCHOP animals, despite widespread activity of the EF-1α promoter. Although we did not detect the presence of FUS–CHOP protein in our double-transgenic FUSxCHOP animals (Figure 1c), the possibility of recombination of FUS and CHOP resulting in expression of fused FUS–CHOP sequences in the double-transgenic FUSxCHOP animals was ruled out by PCR with FUS forward and CHOP reverse primers (data not shown).

We next defined the adipose tissue disease generated in the double-transgenic FUSxCHOP animals. Detailed analysis of the tumor cells established the diagnosis as malignant liposarcomas.

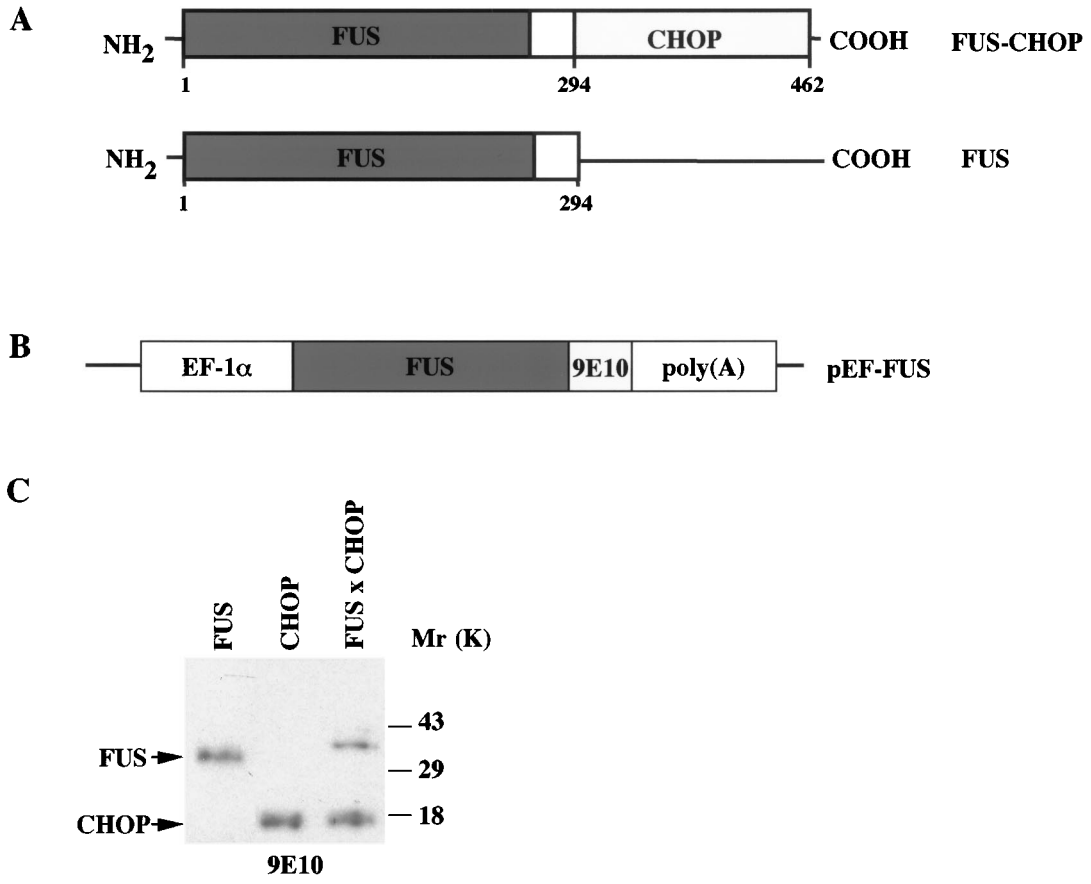

Figure 1 FUS and double FUSxCHOP transgenic mice. (**a**) Schematic representation of the FUS–CHOP protein created as a result of the t(12;16)(q13;p11) chromosomal translocation and the altered form of FUS–CHOP, FUS protein, used in this study. (**b**) The human FUS cDNA containing a tag-sequence for the human c-Myc (amino acids 408–439) at the 3′ end was cloned into the pEF–BOS vector, which contains the EF-1 promoter sequences and polyadenylation and splice signals from the human G-CSF gene, to give pEF–FUS. (**c**) Expression of the pEF–FUS transgene and expression of FUS and CHOP proteins in double FUSxCHOP transgenic mice was determined by Western blot analysis in the fat of a 2-month-old EF–FUS and double FUSxCHOP transgenic, respectively. As a negative control we used the fat from a 2-month-old CHOP transgenic littermate. The location of FUS and CHOP is as indicated

Haematoxilin/Eosin staining showed that the tumor cells had an adipoblast morphology (Figure 3). Histologically, all tumors were diffuse liposarcomas composed of medium to large cells with round nuclei and were very similar to human liposarcomas (see http://www-medlib.med.utah.edu/WebPath/NEOHTML/NEOPLO52.html) and liposarcomas generated in FUS–CHOP transgenic mice (Pérez-Losada *et al*., 2000a). Similarly to FUS–CHOP transgenic mice, the histopathological analyses of the white adipose depots in the double-transgenic FUSxCHOP animals revealed multiple abnormalities. Some contained only modest morphological changes consisting primarily of an increase in nuclear size and number per microscopic field, but the white adipose tissue (WAT) of a majority of the mice had a much more hypercellular appearance and contained scattered cells with enlarged pleomorphic nuclei (Figure 3). Nests of compact anaplastic liposarcoma cells were often scattered throughout the WAT depots. Similarly to FUS–CHOP transgenic mice, WAT deposits in these animals were generally affected at least with abnormal adipocytes and, in some cases, foci of sarcomas were clearly discernible. Pathological changes were not observed in the brown adipose tissue (BAT) of these transgenic mice. Tumor infiltration of non-adipose tissues was usually visible and was confirmed microscopically (Figure 4). These data define the tumors generated in the double-transgenic FUSxCHOP as malignant liposarcomas. We next examined the expression of adipocyte-specific genes in order to define the level at which the differentiation is blocked in FUSxCHOP liposarcomas. Similarly to FUS–CHOP transgenic mice (Pérez-Losada *et al*., 2000a) and despite their block in differentiation each liposarcoma examined was found to express significant levels of PPAR-γ RNA, c/EBPβ, δ and α and aP2 comparable to that of normal fat and FUS–CHOP liposarcomas (data not shown). These results suggest that liposarcomas in the double-transgenic FUSxCHOP mice have been transformed at a point in the differentiation process after induction of PPARγ expression. Moreover, PPARγ is not

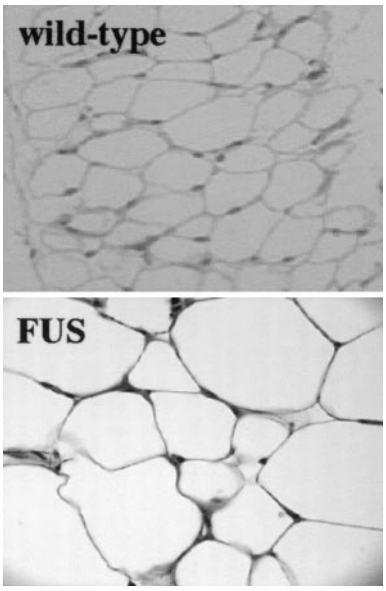

Figure 2 Histologic appearance of the adipose tissues in EF–FUS transgenic mice. Hematoxylin-eosin stained section of the adipose tissues coming from wild-type ($\times 10$) and FUS transgenic mice ($\times 20$)

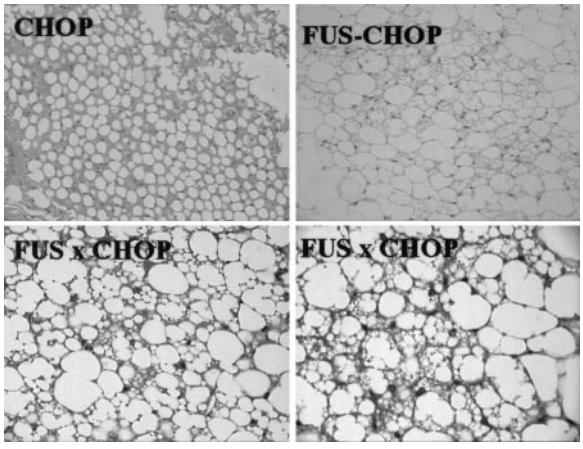

Figure 3 WAT abnormalities in double-transgenic FUSxCHOP animals. Hematoxylin-eosin stained section of the adipose tissue in the region of the testis from a double-transgenic FUSxCHOP mouse shows the effacement of the normal fat architecture compared to control mice. The presence of the lipoblasts are visible in this liposarcoma. Although the liposarcoma disease in FUSxCHOP transgenic mice was very similar to FUS–CHOP mice, the level of adipoblast infiltration revealed by histological analysis was not identical. Similar results were found in multiple sections from double-transgenic FUSxCHOP mice. The original magnification is $\times 10$

expressed in any other type of soft tissue sarcoma (Tontonoz *et al*., 1997) and can be considered a sensitive marker for distinguishing liposarcoma from other histologic types of soft tissue sarcoma. Therefore, the double-transgenic FUSxCHOP mice reproduce the same phenotype with which the *FUS–CHOP* fusion gene is associated both in human pathology and transgenic mice (Pérez-Losada *et al*., 2000a). These

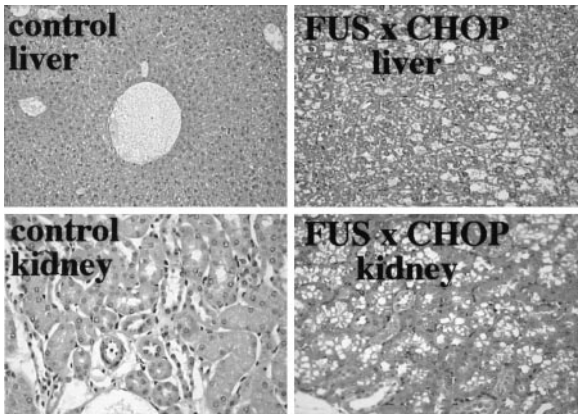

Figure 4 Adipocytic infiltration of liver and kidney in double-transgenic FUSxCHOP mice. Hematoxylin-eosin stained sections of the liver and kidney tissues coming from wild-type and FUSxCHOP transgenic mice

observations provide genetic evidence that FUS and CHOP domains function *in trans* for the mutual restoration of liposarcoma.

Embryonic fibroblasts from FUS mice differentiate into adipocytes but adipocyte differentiation is blocked in embryonic fibroblasts from double-transgenic FUSxCHOP mice

To investigate whether the FUS domain could restore the same adipocyte inhibition capacity than FUS–CHOP in CHOP-expressing cells, we prepared primary embryonic fibroblasts from FUS and double-transgenic FUSxCHOP mice and examined their differentiation properties as previously described (Pérez-Losada *et al*., 2000a). The embryonic fibroblasts from each line were cultured to confluent and then treated with the standard differentiation induction medium. As shown in Figure 5, embryonic fibroblasts from FUS and CHOP mice differentiated into adipocytes and accumulated many lipid droplets in response to hormonal stimulants. In contrast, embryonic fibroblasts from double-transgenic FUSxCHOP mice can hardly differentiate into adipogenic cells and accumulate very few lipid droplets, similarly to FUS–CHOP embryonic fibroblasts (Figure 5). The Oil-Red-O staining demonstrated clear differences in adipocyte phenotype (Figure 5) and, similarly to FUS–CHOP MEFs, MEFs from double-transgenic FUSxCHOP mice only expressed the earliest markers of differentiation (data not shown), which corresponds to pre-adipocytes. These results show that adipocyte differentiation was blocked in cultured embryonic fibroblasts from double-transgenic FUSxCHOP mice but not in MEFs derived from CHOP transgenic mice treated with adipogenic hormons. Interference with the normal process of differentiation contributes to the oncogenic potential of FUS–CHOP fusion protein (Pérez-Losada *et al*., 2000a) and this capacity is restored by the FUS domain into CHOP-expressing cells.

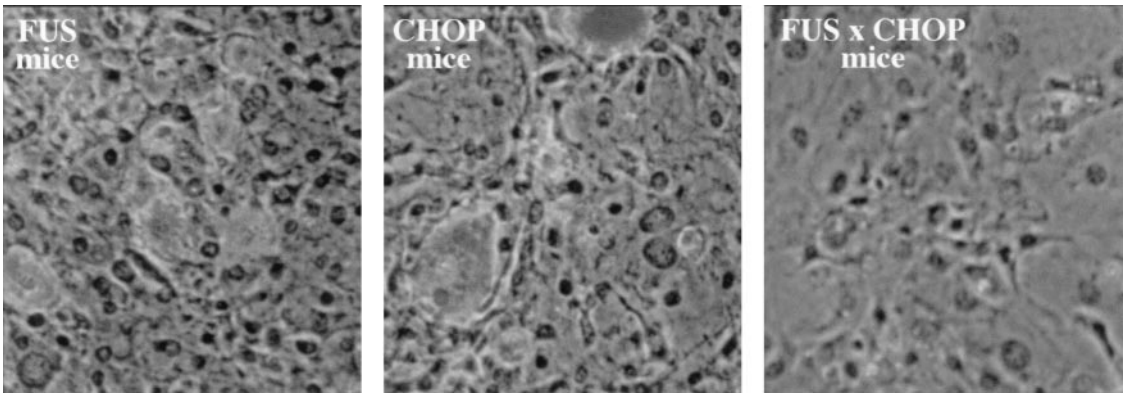

Figure 5 Adipogenic differentiation from primary embryonic fibroblasts expressing altered forms of FUS–CHOP protein. Primary embryonic fibroblasts from each transgenic line were cultured in the presence of standard differentiation induction medium (containing 0.5 mM isobutylmethylxantine, 1 μM dexamethasone, 5 μg/ml insulin and 10% FBS). After 8 days of differentiation, cells were observed by light microscopy with Oil-Red-O staining. Cells from FUS, CHOP and double-transgenic FUSxCHOP mice are shown. The original magnification is $\times 20$. This experiment was repeated three times using cells prepared from all transgenic lines and from different embryos and similar results were obtained

Discussion

We have demonstrated that directing expression of the FUS–CHOP fusion protein to immature mouse cells initiated sarcomas with adipose features (Pérez-Losada *et al*., 2000a). We have similarly expressed altered forms of FUS–CHOP, showing that the truncated protein CHOP which lacks the FUS activation domain does not generate any tumors neither affect the development of WAT *in vivo* (Pérez-Losada *et al*., 2000b). Concordant with these data, adipocyte differentiation was not blocked in cultured CHOP embryonic fibroblasts treated with adipogenic hormones. Thus, these results show that the transcriptional activation by FUS is indispensable for tumorigenesis. However, these results do not clarify whether the relationship between the FUS and CHOP domains is *in cis* or *in trans*. To test the significance of FUS and CHOP domain interactions within a living mouse, we generated mice expressing the FUS domain and crossed them with CHOP-transgenic mice to generate double-transgenic FUSx-CHOP animals. Here we show that expression of the FUS domain restores liposarcoma development in CHOP-transgenic mice. Double-transgenic FUSx-CHOP animals resulted in most of the symptoms of human liposarcomas, including the presence of lipoblasts with round nuclei, accumulation of intracellular lipid, induction of adipocyte-specific genes and a concordant block in the differentiation program. No tumors of other tissues were found in these transgenic mice despite widespread activity of the EF1α promoter, underscoring the relevance of relationship between FUS-CHOP and the cell environment (Sánchez-García, 1997). Our results provide genetic evidence that FUS and CHOP domains function *in trans* for the mutual restoration of liposarcoma and support a model whereby the *FUS–CHOP* fusion gene represents a gain-of-function mutation of both FUS and CHOP that deregulate both gene expression and protein activity. Further studies to identify the target genes and proteins that are regulated by FUS–CHOP using our transgenic mice will produce a clearer picture of the development defect in liposarcoma. In conclusion, our results identify a new molecular mechanism responsible for the genesis of solid tumor-associated fusion genes which might have impact beyond myxoid liposarcoma.

Material and methods

The generation and screening of transgenic mice

PCR amplification of the FUS portion of FUS–CHOP was done to facilitate cloning by adding restriction enzymes using primers that hybridize to the 5′ and 3′ ends of the FUS cDNA. The product was cloned into the M13mp19 vector, added to 3′ end an in-frame sequence coding for a MYC-tag recognized for the 9E10 antibody, and sequenced. The cDNA was cloned into a plasmid, pEF–BOS, containing sequences of the EF1-α promoter, followed by a polylinker region linked to the poly(A) adenylation signal from human G-CSF cDNA as described (Mizushima and Nagata, 1990). Linear DNA fragments for microinjection were obtained by *Hind*III digestion and injected by the INIA Transgenic Facility into CBAxC57BL/6J fertilized eggs. Transgenic founders were identified by Southern-blot analysis through detection of the novel EF-FUS construct from samples of DNA extracted from tails, respectively. CHOP mice has been previously described (Pérez-Losada *et al*., 2000b) and double-transgenic FUSxCHOP animals were generated by crossing FUS and CHOP transgenic mice.

Histological analysis

FUS and double-transgenic FUSxCHOP mice were subjected to standard necropsy. All major organs were closely examined under the dissecting microscope, and samples of each organ were processed into paraffin, sectioned and examined histologically. Tumor specimens from the transgenic mice were fixed into 10% formalin overnight, then

processed, embedded in paraffin, and 6 μm sections were stained with hematoxylin and eosin and photographed. All tissue samples were taken from homogenous and viable portions of the resected sample by the pathologist (T. Fores) and fix within 2–5 min of excision.

Preparation of primary embryonic fibroblasts and induction of adipogenic differentiation

Primary embryonic fibroblasts were harvested from 14.5 d.p.c. embryos. Cells were cultured at 37°C in Dulbecco's modified Eagle's medium (DMEM; Boehringer Ingelheim) supplemented with 10% heat-inactivated FBS (GIBCO/BRL). Cells were plated to 24-well or 60 mm plastic dishes and propagated to confluence. Two days later, medium was replaced with standard differentiation induction medium containing 0.5 mM isobutylmethylxantine (Sigma), 1 μM dexamethasone (Sigma), 5 μg/ml insulin (Sigma) and 10% FBS (GIBCO/BRL). This medium was removed every other day. After 8 days, the appearance of cytoplasmic lipid accumulation was observed by microscopy with Oil-Red-O staining. The Oil-Red-O staining was performed as follows: cells were washed with phosphate-buffered saline (PBS), and then stained with 60% filtered Oil-Red-O stock solution (0.15 g of Oil-Red-O (Sigma) in 50 ml of isopropanol) for 30 min at 37°C. Cells were washed first with 60% isopropanol and then briefly with water and visualized.

Western blot analysis

Single cell suspensions coming from different tissue samples were analysed by immunoblotting procedures essentially as described (Castellanos *et al*., 1997). Lysates were run on a 10% SDS–PAGE gel and transferred to a PVDF membrane. After blocking, the membrane was probed with c-Myc (9E10) mouse monoclonal antibody (Santa Cruz), which is specific for human c-Myc (amino acids 408–439). Reactive bands were detected with an ECL system (Amersham).

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References

Aman P, Panagopoulos I, Lassen C, Fioretos T, Mencinger M, Toresson H, Hoglund M, Forster A, Rabbitts TH, Ron D, Mandahl N and Mitelman F. (1996). *Genomics,* **37,** 1–8.

Batchvarova N, Wang XZ and Ron D. (1995). *EMBO J.,* **14,** 4654–4661.

Carlson SG, Fawcett TW, Bartlett JD, Bernier M and Holbrook NJ. (1993). *Mol. Cell. Biol.,* **13,** 4736–4744.

Castellanos A, Pintado B, Wuruaga E, Arévalo R, López A, Orfao A and Sánchez-García I. (1997). *Blood,* **90,** 2168–2174.

Chang HR, Hajdu SI, Collin C and Brennan MP. (1989). *Cancer,* **64,** 1514–1520.

Crozat A, Aman P, Mandahl N and Ron D. (1993). *Nature,* **363,** 640–644.

Fornace Jr AJ, Nebert DW, Hollander MC-, Luethy JD, Papathanasiou M, Fargnoli J and Holbrook N-J. (1989). *Mol. Cell. Biol.,* **9,** 4196–4203.

Ichikawa H, Shimizu K, Hayashi Y and Ohki M. (1994). *Cancer Res.,* **54,** 2865–2868.

Knight JC, Renwick PJ, Cin PD, Van den Berghe H and Fletcher CD. (1995). *Cancer Res.,* **55,** 24–27.

Mack TM. (1995). *Cancer,* **75,** 211–244.

Mizushima S and Nagata S. (1990). *Nucleic Acids Res.,* **18,** 4322.

Panagopoulos I, Aman P, Fioretos T, Höghind M, Johansson B, Mandahl N, Heim S, Behrendtz M and Mitelman F. (1994). *Genes Chrom. Cancer,* **11,** 256–262.

Panagopoulos I, Mandahl N, Mitelman F and Aman P. (1995). *Oncogene,* **11,** 1133–1137.

Pérez-Losada J, Pintado B, Gutiérrez-Adán A, Flores T, Bañares-González B, Calabia del Campo J, Martín-Martín JF, Battaner E and Sánchez-García I. (2000a). *Oncogene,* **19,** 2413–2422.

Pérez-Losada J, Sánchez-Martín M, Rodríguez-García MA, Pérez-Mancera PA, Pintado B, Flores T, Battaner E and Sánchez-García I. (2000b). *Oncogene,* **19,** 6015–6022.

Prasad DD, Ouchida M, Lee L, Rao VN and Peddy ESP. (1994). *Oncogene,* **9,** 3717–3729.

Price BD and Calderwood SK. (1992). *Cancer Res.,* **52,** 3814–3817.

Rabbitts TH, Forster A, Larson R and Nathan P. (1993). *Nature Genet.,* **4,** 175–180.

Ron D and Habener JF. (1992). *Genes Dev.,* **6,** 439–453.

Sánchez-García I and Rabbitts TH. (1994). *Proc. Natl. Acad. Sci. USA,* **91,** 7869–7873.

Sánchez-García I. (1997). *Annu. Rev. Genetics,* **31,** 429–453.

Shimizu K, Ichikawa H, Tojo A, Kaneko Y, Maseki N, *et al.* (1993). *Proc. Natl. Acad. Sci. USA,* **90,** 10280–10284.

Tontonoz P, Singer S, Forman BM, Sarraf P, Fletcher JA, Fletcher CDM, Brun RP, Mueller E, Altiok S, oppenheim H, Evans RM and Spiegelman BM. (1997). *Proc. Natl. Acad. Sci. USA,* **94,** 237–241.

Wang XZ, Lawson B, Brewer JW, Zinszner H, Sanjay A, Mi LJ, Boorstein R, Kreibich G, Hendershot LM and Ron D. (1996). *Mol. Cell. Biol.,* **16,** 4273–4280.

Zinszner H, Albalat R and Ron D. (1994). *Genes Dev.,* **8,** 2513–2526.